



## Antioxidant Activity of Alkaloid Compounds from *Litsea cubeba* Lour.

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### ABSTRACT

*Litsea cubeba* (Lour.) heartwood. was extracted by maceration. Ethanol extract was fractionated with liquid-liquid extraction using n-hexane, chloroform at pH 3, 7, 9 and 11 to obtained alkaloid fractions. Isolation of alkaloid compounds were isolated with preparative thin layer chromatography. Antioxidant activity for fractions and isolates were determined with DPPH and ABTS assay. The highest IC<sub>50</sub> were given by chloroform fractions at pH 7 and compound III. They IC<sub>50</sub> were 23.81 ± 0.01; 3.12 ± 0.02 µg/mL for DPPH assay and 56.43 ± 0.06; 8.62 ± 0.02 µg/mL for ABTS assay. Compound III was showed maximum absorbance at (302.8; 280.8; 206 nm) and wave number (3286.70; 3109.25; 2939.52; 2835.36; 1589.34; 1462.04; 1365.60; 1246.02; 1018.41; 879.54 cm<sup>-1</sup>) which indicate its an alkaloid compound with hydroxyl, methyl, methylene and methoxy group. The results reveal that alkaloid fractions and compounds of *Litsea cubeba* heartwood has very strong antioxidant potential.


**Keywords:** Antioxidant. *Litsea cubeba*, Heartwood, Alkaloid, Fractions, Compounds.

### INTRODUCTION

Oxidation is an important process (normally) in living organisms, free radicals producing from metabolism pathway process or enviromental sources that interact sustainable with biological system. Reactive species are molecules which have an electronic unstability and most reactive. Reactive oxygen species (ROS) are the biggest sources of primary catalyst which initiate

process of oxidation in vivo and in vitro and produce oxidative stress. Oxidative stress products when reactive forms of oxygen are produced faster than they could be safely neutralized with antioxidant mechanisms and/or from a decrease in antioxidant defense. The uncontrolled production of oxygen free radicals and the unrateabled system of antioxidant capability in protection results in the cause of many diseases, such as cancer, diabetes, heart diseases, Alzheimer's, and aging<sup>1-6</sup>.



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Attarasa (*Litsea cubeba* (Lour.)) is a plant from Lauraceae family which contain much essential oils which used as antidepressant, antiinflammation, antioxidant, pesticide, antimicrobial, anticancer and neuro pharmacology<sup>3</sup>. Methanol extract from attarasa fruits showed to be active on HeLa cell lines which cause apoptosis through activation of caspase 3/7<sup>3,4</sup>. There are more than forty isoquinolin alkaloids that contained in *Litsea* genus which are active as antibacterial agents against *Staphylococcus aureus* ((+)-*N*-(methoxycarbonyl)-*N*-nordicentrin; (+)-*N*-(methoxycarbonyl)-*N*-norpredicentrin; (+)-*N*-(methoxycarbonyl)-*N*-norglaucine)<sup>5</sup>. The heartwoods of *Litsea cubeba* contained high level of phenolic and flavonoid and found to be active as antioxidant<sup>6</sup>. The aim of this study was to determine antioxidant activity of alkaloid fractions and compounds of *Litsea cubeba* Lour, heartwood.

## EXPERIMENTAL

### Plant and chemicals material

Fresh heartwood of *Litsea cubeba* (Lour.) was collected from Balige subdistrict, Sumatera Utara province, Indonesia, *Litsea cubeba* (Lour.) was identified in Herbarium Medanense, Faculty of Mathematic and Natural Products, University of Sumatera Utara, and the voucher specimen was deposited in herbarium. Chemicals used were distilled water (Bratachem), chloroform (Fulltime), hydrochloric acid (Merck & Co.), sodium hydroxide (Merck & Co.), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), 2,2'-azino-bis(3-ethylbenzothiazoline-sulphonic acid (ABTS) (Sigma), methanol (Merck & Co.),

### Preparation of Extract, Fractions and Isolates

The air-dried and powdered heartwoods of *Litsea cubeba* (Lour.) (1 kg) were repeatedly macerated with ethanol 96% (3x3 d, 7.5 L), The filtrate was evaporated to give a viscous extract<sup>7-9</sup>, Viscous extract was fractionated with n-hexane and continue with chloroform at pH 3,7,9 and 11, Chloroform fraction at pH 7 was fractionated with preparative thin layer chromatography (P-TLC) (Merck) using mobile phase dichlormetane: methanol:ammonia (90:10:1) and sprayed with dragendorff reagent<sup>10</sup>. The alkaloid compounds were analyzed with spectrophotometer UV-Vis

(Shimadzu) and FTIR (Shimadzu).

### DPPH radical scavenging activity

The DPPH assay was tested based on previously method<sup>11</sup>, 200  $\mu$ M solution of DPPH• in methanol was added to various concentrations. After 60 min. absorbance was measured at 516 nm and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples<sup>2, 5</sup>.

### ABTS radical anion scavenging activity

The ABTS radical was produced from reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM pottasium persulfate ( $K_2S_2O_8$ ) solution, stored in the dark at temperature room stand for 16 h, Before use, solution was diluted with phospate buffer saline (PBS) to obtain an absorbance of  $0.700 \pm 0.020$  at 734 nm. The fractions and isolates with various concentrations with 1 mL of ABTS solution was homogenized and its absorbance was recorded at 734 nm, PBS blanks were run in each assay and all of measurements were done after at least 6 min. Similarly the reaction mixture of standard group were obtained using quercetin, ABTS scavenging ability was expressed as  $IC_{50}$  ( $\mu$ g/mL) [5].

### Statistical analysis

Data was expressed as mean  $\pm$  SD which were analyzed using the SPSS 21 software.

## RESULTS AND DISCUSSION

### Antiradical activity

Antiradical activity of the plant was measured in term of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple colour in methanol solution, antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character<sup>12</sup>. Antioxidant assay with DPPH and ABTS which is based on the ability of DPPH and ABTS, the stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action<sup>13</sup> and has been largely used as a quick, reliable and reproducible at *in vitro* antioxidant activity assay<sup>14</sup>. The reducing capacity of compounds could serve as marker of potential

antioxidant activity<sup>15-18</sup>. Alkaloids are compound which contain OH and NH functional group, they are could be donating their hydrogen to DPPH and

**Table 1: IC<sub>50</sub> value of alkaloid fractions and compounds of *Litsea cubeba* heartwood with DPPH and ABTS assay (Mean ± SD, 3 times of replication)**

Treatment	IC <sub>50</sub> (µg/mL) DPPH	ABTS
n-hexane Fraction	409.76 ± 0.55	236.15 ± 0.57
Chloroform Fraction pH 3	92.38 ± 0.02	114.52 ± 0.33
Chloroform Fraction pH 7	23.81 ± 0.01	56.43 ± 0.06
Chloroform Fraction pH 9	28.63 ± 0.05	71.73 ± 0.17
Chloroform Fraction pH 11	103.75 ± 0.03	190.77 ± 0.44
Water Fraction	146.46 ± 0.08	149.82 ± 0.39
Compound I	25.92 ± 0.01	21.45 ± 0.02
Compound II	3.12 ± 0.02	8.62 ± 0.02
Compound III	29.75 ± 0.01	19.15 ± 0.04
Quercetin	4.95 ± 0.01	11.39 ± 0.12

ABTS<sup>19</sup>, IC<sub>50</sub> for each fraction and compound shown in Table 1.

#### Identification of compounds

Compounds were isolated from chloroform fraction at pH 7 identification with the aid of spectroscopic methods notably UV-Vis and FTIR spectrophotometer. The UV-Vis spectrum were showed compound I (302.8; 280.8; 206 nm), compound II (282.8; 207.6 nm), compound III (301.8; 281.8; 218.8 nm). The FTIR spectrum were showed compound I (3394.72; 2943.37; 2839.22; 1589.34; 1462.04; 1369.46; 1242.16; 1033.85; 871.82 cm<sup>-1</sup>), compound II (3394.72; 2931.80; 2846.93; 1593.20; 1454.33; 1365.60; 1261.45; 1006.84; 875.68 cm<sup>-1</sup>), compound III (3286.70; 3109.25; 2939.52; 2835.36; 1589.34; 1462.04; 1365.60; 1246.02; 1018.41; 879.54 cm<sup>-1</sup>). The O-H groups were interpreted by wave number between 3000 – 3500 cm<sup>-1</sup> (3394.72 and 3286.70 cm<sup>-1</sup>). The N-H group was showed at

3109.25 cm<sup>-1</sup>. The C-H stretching groups were showed at (2800 – 2950 cm<sup>-1</sup>) and C-H bending groups were showed at (2800 – 2950 cm<sup>-1</sup>). Many researchers were reported that alkaloid compounds have major functional groups O-H, N-H and C-H stretching and bending groups<sup>19, 20, 21</sup>.

#### CONCLUSION

The result of this study showed that alkaloid fractions and compounds of *Litsea cubeba* heartwood have antioxidant activity.

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